

XXV. "An Account of Experiments on the Influence of Colloids upon Crystalline Form, and on Movements observed in Mixtures of Colloids with Crystalloids." By WILLIAM M. ORD, M.D. Lond., F.L.S. Communicated by J. SIMON, C.B., D.C.L., F.R.S. Received June 4, 1879.

[PLATE 1.]

In the "Proceedings of the Royal Society" for 1866, Dr. Montgomery has recorded some very remarkable observations made by him on the behaviour of "myelin" when brought into contact with water. "Myelin" being a term of various applications, it is necessary to state that the substance indicated in Dr. Montgomery's statement is an alcoholic extract of yolk of egg. A small quantity being placed on a microscope-slide, and covered with thin glass, is brought under observation by a tolerably high object-glass. The mass is shapeless, and indistinguishable from crushed grease till the addition of water, when a multitude of minute rounded processes begin to sprout from all parts of the surface exposed to the action of the water. The processes are, when fairly formed, tube-like, with bulbous or simply rounded ends, have a thick, refractile contour, and apparently fluid contents. They remind one of nerve-tubes, or, still more closely, of the tubular and rounded projections which are seen, under the microscope, to issue from the cut ends of nerves in contact with water. Dr. Montgomery shows in the same paper that, if albuminous fluid be used instead of water, spheroidal protrusions are formed, and float free.

Recently, in the examination of a biliary calculus containing much cholesterin, my attention has been drawn to some very remarkable changes of form undergone by that substance under certain conditions—changes so important in their resemblance to phenomena taking place in minute living organisms, that I am induced to give an account of them. It will be seen that they belong partly to the class of changes described by Dr. Montgomery, as above quoted, and partly to those described by Mr. Rainey in his well-known work on "Molecular Coalescence."

Pure cholesterin crystallises, as everybody knows, in the form of colourless rhombic plates. It is insoluble in water and most saline solutions, but is supposed to be held in solution in the bile by taurocholic acid or the soda salt of that acid. In biliary calculi it is found for the most part crystalline; but at the surface of calculi and in biliary gravel it often occurs in lustrous spherules.

Experiment 1.—An ethereal solution of cholesterin is added to fresh egg-albumen in the proportion of one part to two. After good shaking in a test-tube, the mixture becomes gelatinous, and is left for twenty-four hours in the test-tube, or for any period exceeding half an hour, in an evaporating dish to allow of free escape of the ether. As the

ether passes off, the cholesterin is deposited in the substance and on the surface of the albumen. The superficial deposit is partly crystalline, but the deposit in the albumen is entirely spherical or acicular. The crystals on the surface are rarely perfect, and generally have rounded angles (Plate 1, figs. 1, 2). The spheres are of all sizes up to $\frac{1}{500}$ inch diameter, and are at first homogeneous and singularly lustrous (fig. 17). But they speedily begin to undergo internal disintegration. Becoming cloudy, they are soon made out to be composed of fine short needles, packed together like very closely cropped hair, brushed in a direction running from one pole to the opposite (figs. 3—11). Subsequently they break up into tufts of hair-like needles, radiating in a brush from the pole of divergence, and these tufts expand and grow till each of them becomes a long curl or ringlet of wavy hair (figs. 10, 11). By the end of a week nearly all the cholesterin has come to this state of long curling fibrils, and the whole process may remind the observer of the growth of minute confervoid filaments from spores.

A new series of phenomena is observed when the albumen, having the cholesterin immersed in it, is treated with glacial acetic acid. But before describing this change, the behaviour of cholesterin in presence of acids may be described.

Experiment 2.—Treated with nitric acid cholesterin loses its crystalline form with some effervescence, and swells out irregularly at its surface, throwing off oily-looking spherules. Finally it is entirely resolved into such spherules.

Experiment 3.—Treated with hydrochloric acid it undergoes little or no change.

Experiment 4.—On the addition of glacial acetic acid to pure cholesterin, the rhombs are seen under the microscope to undergo an instant change of structure. They break up internally into crystalline fibres, usually springing from one of the angles; and they emit radiating tufts of sharp-pointed needles which seem to grow at the expense of the substance of crystals. If, now, the slide be heated till the acid begins to boil, the cholesterin is wholly or in part dissolved; but, as the slide cools, acicular crystals shoot out from any portion of cholesterin that is left undissolved, or from new centres, and rapidly cover the field. These crystals during their growth give the impression of their being protruded like spears. They are often as thick as the spicules of fresh water sponge, and as refractile. They are solid. The cholesterin appears in this case to be chemically altered by the acetic acid, combining with it to form a sort of ether.

Experiment 5.—Some pure glycerin being added to such a preparation as is produced in the preceding experiment, during cooling, at any point where the glycerin meets the growing spears it breaks them up wholly or partially into globules, making them look as if they had been put into the fire and fused.

Experiment 6.—If glycerin be mixed in about equal quantities with the acetic acid and cholesterin while still on the slide, and if heat be applied, after the covering glass is in place, till bubbles form rapidly, the slide instantly examined is found covered with spheres, some as large as fat cells and quite regular, others egg-shaped, and others aggregated into masses (fig. 12). The masses are dense and very brilliant. As cooling proceeds all the spheres break up. The large ones contract at their surface, and seem thereby to squeeze out fluid from the interior, which takes at first the form of tubes, afterwards of spheres. An active movement of this kind often results in the formation of a group of small spherules in the place of the large one, looking like a glomerulus of spores (figs. 13, 14). Presently these in turn change each instantaneously into needles, the change proceeding as follows:—a single spherule darts away from the mass; as it goes it leaves a stiff raphis in its course, and it is itself proportionately diminished in size, till its last fragment forms the point of the raphis (figs. 15, 16). The ball is, so to speak, unwound into a stiff thread of glassy substance. Here and there spheres break up, without change of place, into variously shaped tufts of needles.

Experiment 7.—Acetic acid is added to cholesterin and albumen, and the mixture is heated to boiling. Examined now as quickly as possible the mixture presents spheres and tufts of needles rapidly growing in the fluid part of the field; spheres and spheroids and combinations of most fantastic shapes in the transparent albumen. The spheres are at first perfectly homogeneous and circular in outline (figs. 17—26). After a time they become oval and gradually a little pointed at the ends. Then usually a little globular protrusion forms at one end or both; then a zone of indentation is drawn round the oval, usually at about one-third of the length of the surface from the protrusion; the interior next gives indication of a subdivision of the substance of the ovoid into two, three, or four mutually compressed spheroids; and at last the whole changes, with magical suddenness, into a crystalline mass of obscurely rhombohedral shape. In process of time this mass breaks up yet further into raphides. The great transformation is attended by upheavals and depressions of the surface, causing a general appearance of singularly life-like convulsions and contortions of the mass. All the change is most rapid during cooling, but it continues slowly for hours and days subsequently till every particle of cholesterin has come to the needle form. If solutions of gelatin be used instead of albumen, similar results are observed. In the gelatin spheres are readily formed, and slowly turn into needles. If acetic acid be added and heat applied the rapid conversion of spheres into needles, attended by active convulsive movements, can be conveniently watched in the cooling mixture; and if glycerin be used as well a great variety of lively transformations may be witnessed.

This series of observations has, in the first place, some importance as involving a rapid mode of exhibiting the influence of colloids upon crystalline form. In Mr. Rainey's original processes, and in the later experiments made by Harting, of Utrecht, and by myself, the formation of spherules of crystalline matter within colloids has been effected only on a microscopic scale, and in the most gradual way. And although Professor Guthrie has recently, in independent and original experiments, obtained large spherical masses of sulphate of copper by the slow evaporation of solutions of that substance mixed with gelatin, it has not been possible to watch so continuously the steps of the process. But here, within a short compass, we are able to see the crystalloid cholesterin assume, when first deposited within the substance of the colloid, perfectly spherical form; we can follow the gradual emancipation of the crystalloid from the control of the colloid and its return to a quasi-crystalline form within the albumen, and to definite crystals within the gelatin or glycerin with acetic acid. We may infer that the crystalloid is not so intensely crystalloid in its tendencies when first deposited as afterwards. We may infer also that the power of the colloid is, under certain circumstances, when coagulation is not produced, intensified by heat; and, in the loss of controlling power experienced by the cooling colloid, we may see a parallel to the loss of power experienced by a degenerating colloid, for instance in the transition from mucous to horny matter. Where the changes are most rapid, as in the heated mixture of cholesterin, acetic acid and glycerin, the transformation is seen to be accompanied by vigorous intestine movements. To review this observation, we have before us in such a mixture, when cooling has only just commenced, perfect lustrous spheres looking like drops of oil. Of a sudden these are affected by peristaltic movements, and, as the surface contracts, the mass is resolved into minute spheres. The heat and the crystallinity have balanced each other for a time, but when the balance has been lost and the process of crystallisation has commenced, the first step has been not the formation of crystals, but of smaller spheres. So long as all was quiet, the large sphere was possible; but when molecular vibrations were set up as the attendants of crystallising separation, the molecules at opposite ends of poles were withdrawn from their power of holding one another in position at such a distance, and the general shaking allowed only of the existence of small spheres, the constituent particles of which were all near enough to each other to allow of each exercising strong enough attractive influence on its fellow to resist the distracting influence of the vibrations set going by the crystallising force. Instead, therefore, of one centre around which, or through which, all attractions were balanced, we have a number of centres with spheres drawn round them proportionate in minuteness to the activity of the perturbing force. The process, in one aspect,

may be compared to the whipping of an egg. In another, it may be compared with the cleavage of the ovum after impregnation. In the ovum up to the time of impregnation the contents of the zona pellucida form one sphere. After impregnation, after the introduction of a material having the power of inducing new activities, shrinkage is followed by movements having the character of a gradual peristalsis, and subsequent fissions reduce the sphere to the mulberry mass of small equal spherules.

Regarded from another point of view, we see a series of movements resulting from the tendency of matter to come to rest. We see stages which may be compared to the spring of the bow when the drawn string is let go; and, again, to the straightening of the unstrung bow. Or we may see in the chain of movements, beginning with the formation of the sphere and ending in the crystal at rest, a parallel to the chain of force formed when water is raised by the sun to the clouds and hill-tops to descend in rain or rivers to the sea—to rest; or to the chemical chain in which oxygen and carbon, torn asunder by light within vegetables, slowly recombine carbonic acid within animals, and in turn come to rest.

Here, again, the question is suggested to me, how far may the simpler movements of the constituents of animal and vegetable organisms be due (among other things) to the struggle of one group of substances against the grasp of another, to the tendency of each substance to ultimate complete separation from the substances with which it is intermixed, and to the tendency of each separated substance to assume the nearest possible approach to a static condition?

The observations have, however, an immediate practical relation to the structure of such biliary calculi as are composed chiefly of cholesterin. Such calculi are seen in sections to be composed of several distinct stratifications. Most commonly they have (1) a thin rugged outer layer, deeply tinged with bile colour; within this (2) a brown portion marked with concentric lines corresponding to laminae; within this (3) a lighter-coloured layer, presenting radiating markings or distinct radiating fissures between crystalline columns; and, lastly (4), at the centre a mass of deep colour and friable consistence (fig. 37). The strata are not sharply defined from each other, but are joined by transitional layers. They differ severally in minute structure. In the outer crust (1) distinct independent spherules and spheroidal masses composed of cholesterin, mixed with mucus and bile-pigment, can be seen (figs. 27—31). In the concentric layers (2) the microscope shows a substance pretty uniformly inter-penetrated by, and partially coloured with, pigment; it is marked with radiating crystalline fibrations of varying degrees of fineness running sinuously at right angles to the concentric markings; in fact, it shows a combination of concentric and radiating arrange-

ment. The light radiating layer (3) is composed of nearly colourless crystalline columns of cholesterin radiating as from the centre of the calculus, with accumulations of pigment in the spaces between them; while the centre (4) is nearly all deep brown pigment, actually black in some cases, mixed here and there with perfectly formed, thick, colourless, rhombohedral slabs of cholesterin. The explanation of this composite structure appears tolerably easy by the light of the experiments recorded. The cholesterin is first deposited in the pigment with or without some mucus. These, the colouring matter in particular, are substances of high molecular composition, and when first secreted in the bile are most typical colloids. But when kept outside the body, or retained for any length of time within it, the bile-pigments slowly exchange the colloid for the crystalline state, and may often be seen in rhombohedral forms of great beauty in hydatid cysts in the liver. In proportion as the pigment is relatively abundant at the time of the precipitation of the cholesterin the latter is more completely fused and intermixed with it. The superficial part of the calculi, of course the latest deposit, shows this intermixture. The concentric layers show a commencement of rearrangement of particles subsequent to deposit. Here the cholesterin and pigment are beginning to separate from each other, and the lines of crystallisation are already related radially to the centre of the calculus. The calculus exhibits on a large scale the mingling concentric and radiant arrangement of molecules seen in the small spheres of carbonate of lime and other salts formed by Mr. Rainey, and in the small spheres found on the surface of the calculi under investigation (see fig. 30). In the light-coloured radiating layer (3) separation has gone further; crystalline fibrillation has been exchanged for strongly-marked angular and columnar form, and the colour is nearly all interstitial to the crystals, while the whole arrangement is again clearly related to the centre of the whole sphere. Lastly, in the very centre (4), where all lines of attraction are balanced, the pigment accumulates in quantity, and the few crystals left are independent and typically perfect in form. The result of the investigation is to the remarkable effect that a metamorphic change is in process within the calculus from the time of its first formation up to the time at which it is submitted to examination, involving a constant rearrangement of particles in relation to the centre of the whole mass, and dependent upon the double tendency seen in cholesterin under experiment: first, the tendency to be deposited in the spherical form when precipitated in a colloid matrix; second, the tendency to revert, in process of time, to a crystalline form: the molecules in their passage from the one state to the other being caught, as it were, by the common attraction of the great sphere, and brought to a certain extent under their dominion. The fact that the colloid forming the matrix is one which tends ultimately to become

crystalloid is probably one reason why the metamorphic changes are more rapid and are pushed further than in urinary calculi.

There is, of course, a considerable variety in the proportions of the various structures in these biliary calculi. Sometimes a gallstone shows entirely a radiating structure. It is then usually but little stained with pigment. Sometimes it is nearly all concentrically marked; the pigment is then abundant. I have throughout spoken more of the pigment than of the mucus as the modifying colloid, since the examination certainly brings it into greater prominence; just as I have elsewhere shown that the chemically allied pigment of urine exercises a parallel effect upon the form of uric acid, by preventing that substance from assuming the rectangular form proper to it in its pure state. The calculus is to all intents and purposes a dead thing within the living body, yet it is of great interest to consider that it is the seat of slow metamorphic changes, and that these changes are effected in and by substances characteristically belonging to animal life, and that they simulate processes seen in living structures, processes that have been regarded as purely vital and dependent upon the influence of cells or nuclei.

I have next to speak of some allied phenomena exhibited by uric acid. That substance, under certain modes of treatment, manifests a succession of colloid and crystalloid states and attributes parallel on the one side with those of the colloid pigment on the other with those of the crystalloid cholesterolin.

Experiment 8.—If some uric acid be boiled for some time with strong solutions of chloride of sodium or of phosphate of soda, and the solutions be left after filtration to crystallise, urate of soda is deposited. But not in crystals. The fluid, after being thoroughly cooled, is seen to be nearly filled with a semi-transparent gelatinous-looking deposit, reminding one of the mucus separated from the urine of early vesical catarrh. This deposit consists, as seen under the microscope, of a gelatinous-looking matrix through which a number of bright but as yet formless points are scattered. After a few hours a slight diminution of bulk is apparent, and the microscope then shows spherical arrangements of bright granules, and bright spherical tracts without structure suspended within the jelly, the bright granules being still present. Slowly, from day to day, the bulk of the precipitate grows less, and it gradually assumes a whitish instead of a transparent aspect. The spherical bodies are gradually resolved into spherical tufts of needles radiating from a common centre; the matrix becomes more and more granular. At last only a little white powder is left lying at the bottom of the liquid; it contains no trace of matrix, and consists entirely of needles, some in tufts, some loose. This condition is not attained in temperate weather before the end of a fortnight or three weeks.

The whole phenomenon suggests at first sight that a strong crystalline solution can act on another crystalloid deposited within it, just as a colloid would act. But the microscopic examination demonstrates that the urate is deposited at first in the form of a colloid, and in its subsequent metamorphoses, which are gradual and distributed, acts in one part as a colloid, in another as a crystalloid. Uric acid is known to be thrown down by acids in what has been called a hydrated form, which is clearly also a colloidal form, and can be seen to be at first structureless, though resolved speedily into crystals. Urate of soda appears also to have its colloidal stage, in ordinary conditions of short duration, but prolonged by the action of the strong saline solution. The explanation then is, that the saline solution retarding metamorphosis allows of a long colloid stage, during which the slowly-formed crystals are modified by that part of the substance which remains still colloidal.

It appears to me that by means of this sort of process the existence of a colloid (nascent) stage in the natural history of many crystalloid substances of high molecule may be sought for and studied. In this way it can be shown that uric acid, when deposited from solutions of urea of certain strength, passes through colloid and spherical stages. With solutions of less than 4 per cent. strength of urea, uric acid, if deposited from the boiling solution, forms fine rectangular crystals. But if solutions of 5 per cent. strength and upward be used, the gelatinous deposit and the spheres are abundantly produced. When I noticed this for the first time, I thought it might be urate of ammonia, resulting from the decomposition of some of the urea. But as it was deposited from acid solutions this was not probable. I then collected a considerable quantity of the deposit, and submitted it to organic analysis. My friend, Mr. John Moss, F.C.S., made several determinations for me; but they did not yield any definite results. And as the deposit occurs with solutions of very decided acidity, I have been inclined to give up a second hypothesis which I had formed—that the spheres were urate of urea. If they be uric acid, it is noteworthy that the colloid stage is shorter than that of urate of soda in the saline solutions.

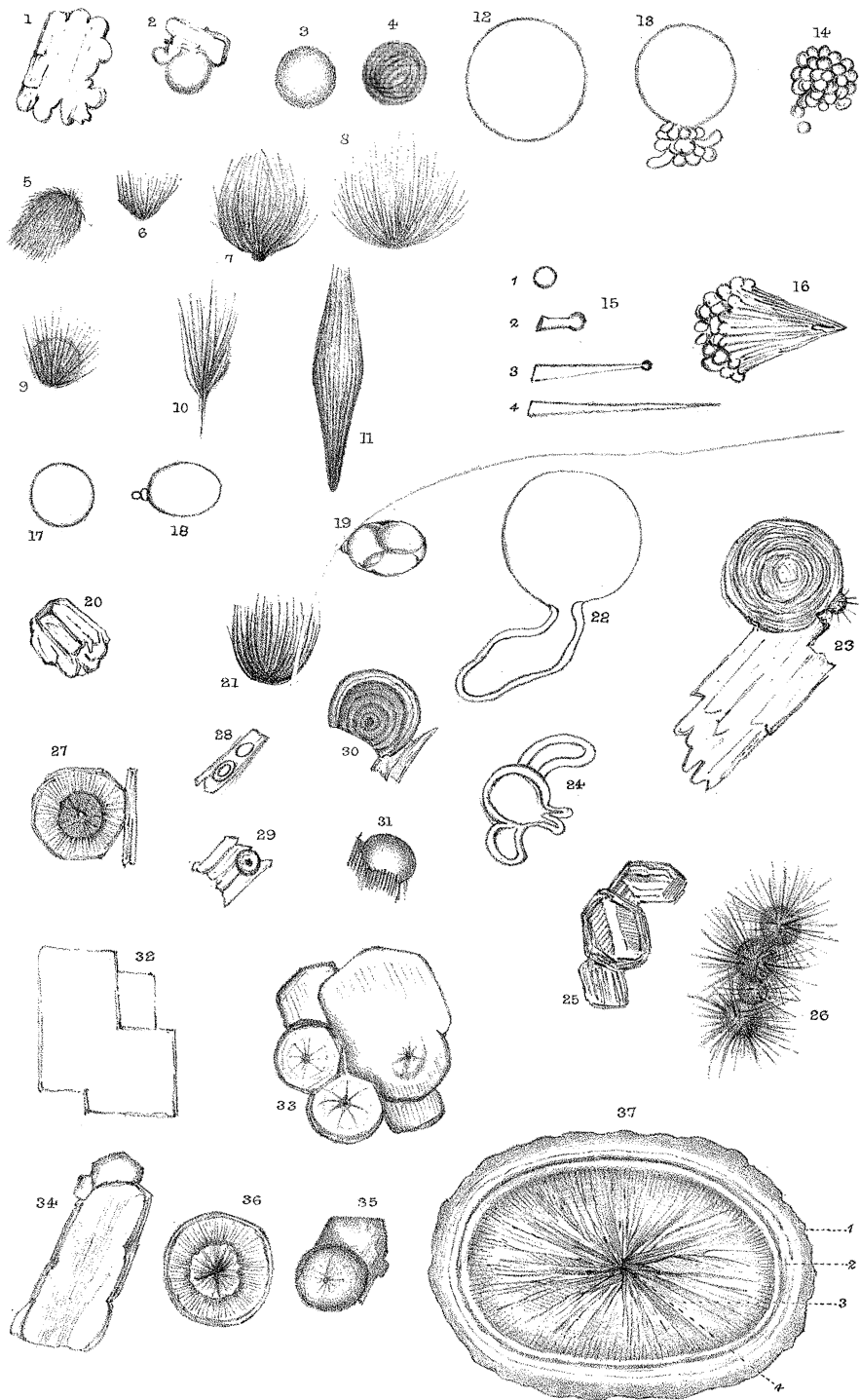
I have already alluded to Dr. Guthrie's masses of sulphate of copper as illustrating on a large scale Mr. Rainey's original observations. They are, however, formed but slowly. I am now able to bring before the Royal Society an experiment, in which spheres of crystalloid matter can be rapidly formed, and of such a size as to be readily visible to the naked eye.

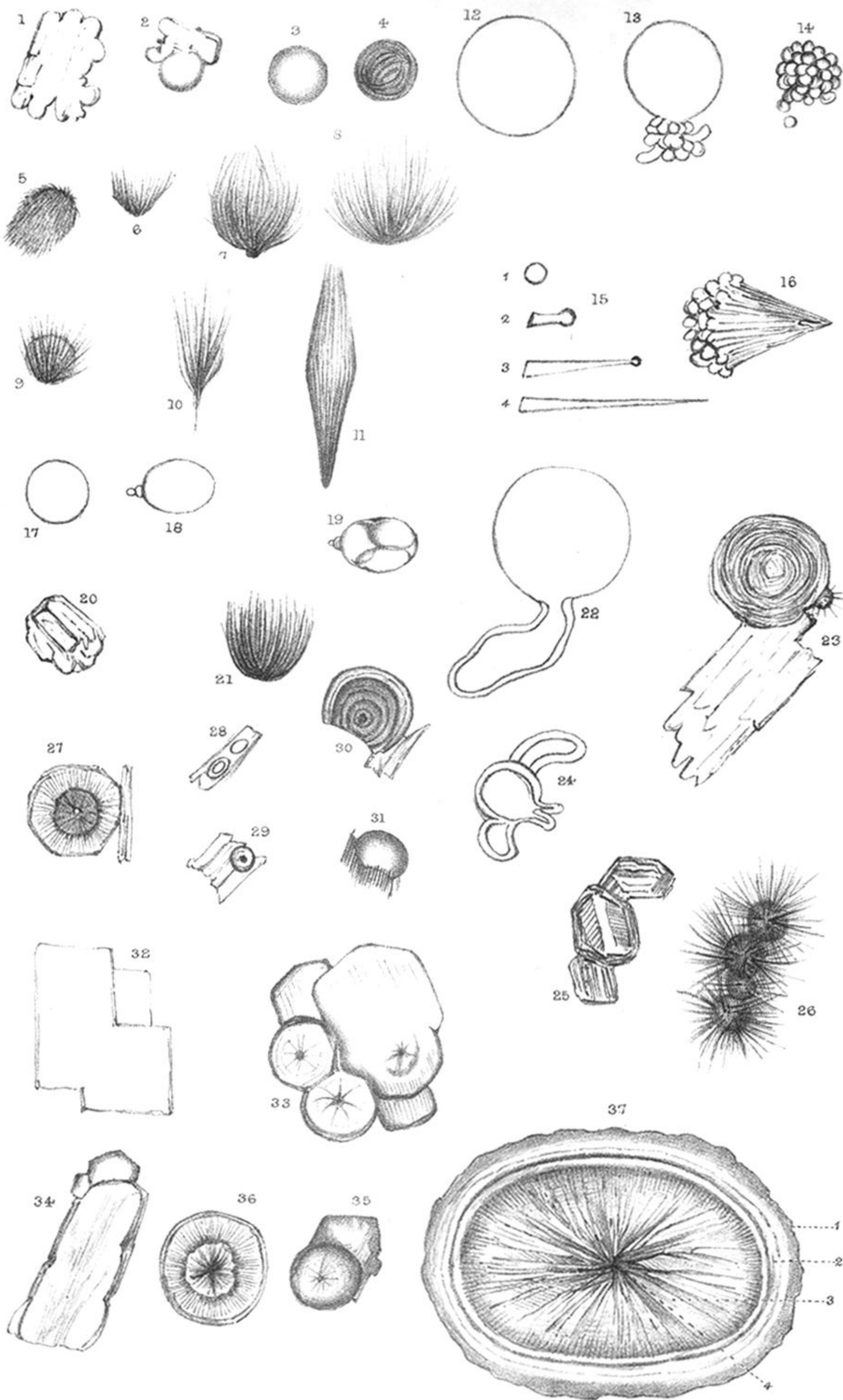
Experiment 9.—If to a solution of urea of from 1020 to 1025 specific gravity strong nitric acid be added in equal bulk, the mixture, at first hot, deposits on cooling to 50° F. crystals of nitrate of urea in the form of separate and distinct brilliant flakes, which subside at once.

Experiment 10.—If a little albumin be added to the solution of urea and if then to the filtered liquid the equal bulk of nitric acid be added as rapidly as possible, the albumin is only partially precipitated, and, after a little warming, is all re-dissolved. In the resulting clear straw-coloured liquid there appear during cooling spherical bodies, suddenly suspended at various depths. They look very much like little globules of solidified fat, and grow rapidly from the size of a mustard-seed to the bulk of a pea. Each is composed of crystalline fibres radiating from the centre, and whereas the deposit in the previous experiment does not occupy more than one-third of the fluid, these fill the whole bulk of the fluid and, mutually compressing each other, give the contents of the tube in which the experiment is made the appearance of a bar of beautiful fibrous spar. The experiment occurred to me first in the examination of concentrated albuminous urine, but it succeeds equally well with pure solutions, and forms, I submit, the most brilliant illustration of Mr. Rainey's valuable work hitherto made known.

DESCRIPTION OF PLATE 1.

- Figures 1 to 11. Cholesterin in albumin : 1, 2, on surface ; 3-11, successive stages of forms observed in the cholesterin deposited within the substance of the albumin.
- Figures 12 to 16. Cholesterin after being heated with acetic acid and glycerin : 12, sphere first formed ; 13, sphere in act of contracting and exuding smaller spheres and tubular processes ; 14, mass of globules like mulberry resulting from subdivision of sphere ; 15, 1, 2, 3, 4, diagrammatic representation of transformation of globule into raphis ; 16, a mulberry mass in act of transformation into a fan of raphides.
- Figures 17 to 26. Shows three kinds, 17-21, 22 and 23, 24-26, of metamorphic change accompanied by movements undergone by cholesterin after heating with acetic acid and albumin.
- Figures 27 to 31. Spheres of cholesterin mixed with crystalline matter, from the surface of a biliary calculus.
- Figures 32 to 36. Forms seen after solution of a calculus in ether and slow evaporation of the liquid in contact with the pigment. They are transitional from crystal to sphere.
- Figure 37. Diagrammatic section of Biliary Calculus. 1, outer crust dark-coloured without either concentric or radiating structure ; 2, second concentric layer ; 3, radiating crystalline layer ; 4, centre, pigmentary, structureless.





DESCRIPTION OF PLATE 1.

Figures 1 to 11. Cholesterin in albumin : 1, 2, on surface ; 3-11, successive stages of forms observed in the cholesterin deposited within the substance of the albumin.

Figures 12 to 16. Cholesterin after being heated with acetic acid and glycerin : 12, sphere first formed ; 13, sphere in act of contracting and exuding smaller spheres and tubular processes ; 14, mass of globules like mulberry resulting from subdivision of sphere ; 15, 1, 2, 3, 4, diagrammatic representation of transformation of globule into raphis ; 16, a mulberry mass in act of transformation into a fan of raphides.

Figures 17 to 26. Shows three kinds, 17-21, 22 and 23, 24-26, of metamorphic change accompanied by movements undergone by cholesterin after heating with acetic acid and albumin.

Figures 27 to 31. Spheres of cholesterin mixed with crystalline matter, from the surface of a biliary calculus.

Figures 32 to 36. Forms seen after solution of a calculus in ether and slow evaporation of the liquid in contact with the pigment. They are transitional from crystal to sphere.

Figure 37. Diagrammatic section of Biliary Calculus. 1, outer crust dark-coloured without either concentric or radiating structure ; 2, second concentric layer ; 3, radiating crystalline layer ; 4, centre, pigmentary, structureless.